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Identification and Quantitative Analysis of β-sitosterol Oxides in Oil of 2 Macrotermitinae Varieties from Congo

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Abstract: Termites are one of the edible insects in the Congo, but very little scientific data are available on this food. Sitosterol oxides are present in vegetable food. However, as cholesterol oxides, biological utility and food safety of some sitosterol oxides are controversial and not clarified. In a chemical study, sitosterol oxides were identified and quantitatively determined in oil of *Bellicositermes natalensis* Haviland from two regions of the Congo by gas chromatography (GC) and Mass spectrometry (MS) analysis. The following β-sitosterol oxides have been found in oil of termites in various amount: 5,6β-epoxysitosterol, 7α-hydroxysitosterol, 7β-hydroxysitosterol, 5α,6β-dihydroxysitosterol, 5,6α-epoxysitosterol and 7-ketositosterol. Total sitosterol oxides amount found was 29.1 μg g^{-1} for termites dried by sun exposure and 39.1 μg g^{-1} for smoked termites. These results showed also that oil of termites contained more sitosterol oxides than edible vegetable oils previously reported. On the other hand this study demonstrated for the first time that phytosterol oxides may be present in some animal oils.

Key words: Lipid, phytosterol oxides, Macrotermitinae, *Bellicositermes natalensis*

INTRODUCTION

β-sitosterols are reported to be the main component in phytosterols fraction of unsaponifiables in vegetable oils. Many researchers are interesting on phytosterols because of their biological activities and their use in some food, as they are useful for health (Ling and Jone, 1995; Moghadasian, 2000; Pioronen *et al*., 2000; Zhang *et al*., 2005a, b, 2006a; Mortuza, 2006; Fernandez-Cuesta *et al*., 2012). It has been shown, *in vitro*, that some cholesterol oxides may be cytotoxic, mutagenic, atherogenic and carcinogenic (Schroepfer Jr., 2000; Guardiola *et al*., 2002). It is also well known that, molecular structures of cholesterol and phytosterol are highly similar. That's how, many and various studies were undertaken on phytosterols and their oxides. The main objectives of these researches were: identification, quantitative determination and biological evaluation of their activities (Guardiola *et al*., 2002, 2004; Lea *et al*., 2004; Roussi *et al*., 2005; Liang *et al*., 2011; Garcia-Liatas and Rodriguez-Estrada, 2011). For example, some biological studies have been done on phytosterol oxides concentrate from which, genotoxicity and subchronic toxicity were not demonstrated (Lea *et al*., 2004). Abramsson-Zetterberg *et al*. (2007) reported a study on phytosterol oxides from a mixture of sitosterol and campesterol, therein, no genotoxic effect was observed *in vivo* in mice. Recently, a study of sitosterol oxides, namely 7-ketositosterol, 7β-hydroxysitosterol, 7α-hydroxysitosterol, a mixture of 6β-hydroxy-3-ketositosterol and 6α-hydroxy-3-ketositosterol has been reported. In this work, no mutagenic activity, towards *Salmonella* Typhimurium strains, was observed (Koschutnig *et al*., 2010).

Although, the above mentioned literatures are favourable for no toxicity from sitosterol oxides, some controversial biological effects have been reported. Koschutnig *et al*. (2009) reported cytotoxic and apoptotic activities of some β-sitosterol oxides. Differences were observed by comparing of activities of 7-ketositosterol, 7β-hydroxysitosterol, 7α-hydroxysitosterol and some mixtures of others β-sitosterol oxides. In this study, 7-ketositosterol and 7α-hydroxysitosterol were found to be more active than the others. On the other hand, Liang *et al*. (2011) working with male hamsters, reported effects of β-sitosterol, stigmasterol and their corresponding oxides in lipoprotein profile and aortic function. These authors found favorable effects for β-sitosterol and stigmasterol but not favorable for both corresponding oxidation products.

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Zhang *et al*. (2006b) reported that sitosterol oxides in oil content can change under sunlight exposure, from 9.6 to 1007 μg g^{-1} in sunflower oil, from 0.8 to 895 μg g^{-1} in soybean oil, from 0.1 to 676 μg g^{-1} in olive oil and 1.9 to 2421 μg g^{-1} in rapeseed oil. The same oil showed variation of total sitosterol oxides from 9.6 to 354 μg g^{-1} for sunflower oil, 0.8 to 331 μg g^{-1} for soybean oil, 0.1 to 294 μg g^{-1} for olive oil and 1.9 to 1083 μg g^{-1} for rapeseed oil, under UV light exposure.

Having said that, further researches are required for a better understanding, of the influence of plant sterols and their oxides on consumers' health. In a previous study, composition of oil, from termites from Congo, has been reported (N'Goka and Julien-David, 2012). This animal oil was comparable to vegetable olive oil taking into account their monounsaturated components. These findings encouraged us to generate complementary information for this oil. The present study reports identification and quantification of β-sitosterol oxides in oil of *Bellicositermes natalensis* Haviland from two regions of the Congo.

MATERIALS AND METHODS

Samples: The termites are the same species of Bellicositermes natalenis Haviland obtained from two origins: Sangha and Plateaux-Cuvette, respectively named termites S and termites PC. A sample was bought at the market in Brazzaville Congo on 2005 September. Termites were transported to the laboratory where they were conserved at -18°C.

Extraction of *Bellicositermes natalenis* oil: Three individual 10 g samples of dry termites of each origin were refluxed with 300 mL of chloroform in weighed flasks using a Soxhlet apparatus according to AACC (1987) method. The fat matter of the termites has been extracted with the help of a Soxhlet equipped with extraction cartridges in cellulose. The termites ground, with the help of a homogenizer to knives, have been submitted to an extraction under ebullition of chloroform during 9 h. The oils were recovered by distilling the solvent in a rotary evaporator at 45°C, then dried to constant weight in a vacuum oven at 90°C for 1 h and weighed.

Extraction of phytosterol oxides: Extraction of phytosterol oxides was carried out as previously described (Zhang *et al*., 2005b), taking advantage of the previously synthesized standards of phytosterol oxides (Zhang *et al*., 2005a). Briefly, a 200 mg oil sample was spiked with a 19-hydroxycholesterol solution (20 μL, 1 mg mL^{-1} in ethyl acetate) as an internal standard. After removal of the solvent with a gentle flow of nitrogen, the sample was dissolved in a mixture of ethanol (9 mL) and a saturated aqueous KOH solution (0.5 mL) before it was submitted to an overnight (15 h) soft saponification at room temperature. The unsaponifiable fraction was extracted with diethyl ether and the oxides were separated from the matrix using a Solid-phase Extraction (SPE) silica gel cartridge and converted to Trimethylsilyl (TMS) ethers with pyridine (50 μL) and N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA), 40 μL. Extraction steps were done in brown bottles and at room temperature. Analysis of 1 μL samples was carried out using a GC-MS chromatograph as described below.

Gas chromatography-mass spectrometry (GC-MS) analysis: GC-MC analysis was carried out as described by Zhang *et al*. (2005b). GC-MS analyses were performed on a Varian Star 3400 GC instrument equipped with an on-column SPI injector coupled to a Varian Saturn 2000 mass sensitive detector (Varian, France) operating in the Electron Impact (EI) ionization mode at 70 eV and monitored on the full-scan range (m/z 40-600). Data acquisition and processing and instrumental control were performed by Varian Saturn WS software. Analytes were separated in a VF-5 ms capillary column (phase stationary: 5% phenyl-95% dimethyl polysiloxane, thickness of 0.1 μm, 60 m×0.25 mm, Varian, France). The column temperature gradient was programmed from 105°C (hold for 2 min) to 170°C at 20°C min^{-1} and then, to 320°C at 7°C min^{-1} (hold for 15 min). The injector operating conditions were as follows: injection volume 1 μL; initial injector temperature of 105°C was increased to 300°C at 100°C min^{-1} (hold for 40 min). Helium (purity 99.9995%) was used as a carrier gas with a flow rate of 1 mL min^{-1}.

Quantitative data were carried out using Selected Ion Monitoring (SIM) analysis. The analytes were quantified as previously reported (Zhang *et al*., 2005b).

RESULTS AND DISCUSSION

Identification and quantitative determination of β-sitosterol oxides were carried out in oil of *Bellicositermes natalensis* Haviland from two regions of the Congo. The total amount of their sitosterol oxides varied from 29.1 to 39.1 μg g^{-1} of oil from these edible insects. This animal oil has proved to contain phytosterol oxides which are generally present in vegetable matter. β-sitosterol oxides composition of the oil and their amount in μg g^{-1} are presented in Table 1. Sitosterol oxides in oil of termites found were: 5,6β-epoxysitosterol, 10.3 and 12.5 μg g^{-1}; followed by 7α-hydroxysitosterol, 6.4 and 10.0 μg g^{-1}; 7β-hydroxysitosterol, 5.2 and 9.3 μg g^{-1};

Table 1: Concentration of β-sitosterol oxides in oil of termites from two origins

Sitosterol oxides (μg g^{-1})	Termites PC	Termites S
7α-hydroxysitosterol	6.4±1.2	10.0±2.00
7β-hydroxysitosterol	5.2±1.1	9.3±2.53
5,6β-epoxysitosterol	10.3±1.5	12.5±3.00
5,6α-epoxysitosterol	2.0±0.4	4.0±1.50
5α,6β-hydroxysitosterol	3.2±1.0	4.3±1.10
7-ketositosterol	2.0±0.7	3.3±0.70
Total amount	29.1	39.1

Values are Mean±SD

5α,6β-dihydroxysitosterol, 3.2 and 4.3 μg g^{-1}; 5,6α-epoxysitosterol, 2.0 and 4.0 μg g^{-1} and 7-ketositosterol, 2.0 and 3.3 μg g^{-1}, respectively for termites PC and termites S. This finding was already observed in edible vegetable oils by Zhang *et al*. (2005b, 2006b). Therein the authors showed that β-sitosterol oxides were present and total amount was reported to be 9.6 μg g^{-1} for sunflower oil, 0.8 μg g^{-1} for soybean oil, 0.1 μg g^{-1} for olive oil and 1.9 μg g^{-1} for rapeseed oil. In the animal material, the subject of this study, the total amount of β-sitosterol oxides was 29.1 μg g^{-1} for termites PC and 39.1 μg g^{-1} for termites S. Termites S contained more oxidized phytosterol then termites PC. This observation suggested that smoking procedure occurred with more oxidation than sun drying. Previous studies of experimental oxidation of edible oils have been reported. Zhang *et al*. (2005b) have been shown that temperature could increase β-sitosterol oxides in oils. More oxidizing compounds were found under sunlight exposure than under UV light exposure, Zhang *et al*. (2006b). The present study showed that termite's oil contained more β-sitosterol oxides (29.1 to 39.1 μg g^{-1}) than edible vegetable oils previously reported. The reason of variation between the chemical compositions of oils of termites PC and S may depend on climatic, geographic conditions, harvest drying procedure.

In our knowledge, sitosterol oxides, therefore phytosterols, are reported for the first time in oil of animal. The high amount of these oxides and the high importance of their biological properties showed that, oil of termites must be chemically characterized and biologically evaluated. Further study must be done to determine phytosterol and phytosterol oxides in fresh termites for a comprehensive study.

CONCLUSION

5,6β-epoxysitosterol, 7α-hydroxysitosterol, 7β-hydroxysitosterol, 5α,6β-dihydroxysitosterol, 5,6α-epoxysitosterol and 7-ketositosterol were identified and quantitatively determined in oil of *Bellicositermes natalensis* Haviland. This study demonstrated for the first time that phytosterol oxides may be present in some animal oils. Under normal conditions of production and conservation, it has been shown that termites contained more sitosterol oxides than edible vegetable oils previously reported. Large amount of these compounds in termites suggested that much attention must be done for evaluation of risk because biological effects and safety aspects of phytosterol oxides are still unclear.

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REFERENCES

AACC, 1987. AACC international approved methods: AACCI Method 46-11.02. American Association of Cereal Chemists Inc., St Paul, MN., USA.

Abramsson-Zetterberg, L., M. Svensson and L. Johnsson, 2007. No evidence of genotoxic effect *in vivo* of the phytosterol oxidation products triols and epoxides. Toxicol. Lett., 173: 132-139.

Fernandez-Cuesta, A., A. Nabloussi, J.M. Fernandez-Martinez and L. Velasco, 2012. Tocopherols and phytosterols in sunflower seeds for the human food market. Grasas Aceites, 63: 321-327.

Garcia-Liatas, G. and M.T. Rodriguez-Estrada, 2011. Current and new insights on phytosterol oxides in plant sterol-enriched food. Chem. Phys. Lipids, 164: 607-624.

Guardiola, F., P.C. Dutta, R. Codony and G.P. Savage, 2002. Cholesterol and Phytosterol Oxidation Products: Analysis, Occurrence and Biological Effects. AOCS Press, Champaign, Pages: 394.

Guardiola, F., R. Bou, J. Boatella and R. Codony, 2004. Analysis of sterol oxidation products in foods. J. AOAC Int., 87: 441-466.

Koschutnig, K., S. Heikkinen, S. Kemmo, A.M. Lampi, V. Piironen and K.H. Wagner, 2009. Cytotoxic and apoptotic effects of single and mixed oxides of β-sitosterol on HepG2-cells. Toxicol. *In vitro*, 23: 755-762.

Koschutnig, K., S. Kemmo, A.M. Lampi, V. Piironen, C. Fritz-Ton and K.H. Wagner, 2010. Separation and isolation of β-sitosterol oxides and their non-mutagenic potential in the Salmonella microsome assay. Food Chem., 118: 133-140.

Lea, L.J., P.A. Hepburn, A.M. Wolfreys and P. Baldrick, 2004. Safety evaluation of phytosterol esters. Part 8. Lack of genotoxicity and subchronic toxicity with phytosterol oxides. Food Chem. Toxicol., 42: 771-783.

Liang, Y.T., W.T. Wong, L. Guan, X.Y. Tian, K.Y. Ma, Y. Huang and Z.Y. Chen, 2011. Effect of phytosterols and their oxidation products on lipoprotein profiles and vascular function in hamster fed a high cholesterol diet. Atherosclerosis, 219: 124-133.

Ling, W.H. and P.J. Jone, 1995. Dietary phytosterols: A review of metabolism, benefits and side effects. Life Sci., 57: 195-206.

Moghadasian, M.H., 2000. Pharmacological properties of plant sterols: *In vivo* and *in vitro* observations. Life Sci., 67: 605-615.

Mortuza, M.G., 2006. Tocopherol and sterol content of some rapeseed/mustard cultivars developed in Bangladesh. Pak. J. Biol. Sci., 9: 1812-1816.

N'Goka, V. and D. Julien-David, 2012. Determination of triacylglycerols, fatty acids and total lipid content in oil of 2 *Macrotermitinaes* varieties from congo. Am. J. Food Technol., 7: 238-244.

Pioronen, V., D.G. Lindsay, T.A. Miettinen, J. Toivo and A.M. Lampi, 2000. Plant sterols: Biosynthesis, biological functions and their importance to human nutrition. J. Sci. Food Agric., 80: 939-966.

Roussi, S., A. Winter, F. Gosse, D. Werner and X. Zhang *et al*., 2005. Different apoptotic mechanisms are involved in the antiproliferative effects of 7beta-hydroxysitosterol and 7beta-hydroxycholesterol in human colon cancer cells. Cell. Death Differ., 12: 128-135.

Schroepfer Jr., G.J., 2000. Oxysterols: Modulators of cholesterol metabolism and other processes. Physiol. Rev., 80: 361-554.

Zhang, X., D. Julien-David, M. Miesch, P. Geoffroy and F. Raul *et al*., 2005a. Identification and quantitative analysis of β-sitosterol oxides in vegetable oils by capillary gas chromatography-mass spectrometry. Steroids, 70: 896-906.

Zhang, X., P. Geoffroy, M. Miesch, D. Julien-David, F. Raul, D. Aoude-Werner and E. Marchioni, 2005b. Gram-scale chromatographic purification of β-sitosterol Synthesis and characterization of β-sitosterol oxides. Steroids, 70: 886-895.

Zhang, X., A. Cambrai, M. Miesch, S. Roussi, F. Raul, D. Aoude-Werner and E. Marchioni, 2006a. Separation of Δ5- and Δ7-phytosterols by adsorption chromatography and semipreparative reversed phase high-performance liquid chromatography for quantitative analysis of phytosterols in foods. J. Agric. Food Chem., 54: 1196-1202.

Zhang, X., D. Julien-David, M. Miesch, F. Raul and P. Geoffroy *et al*., 2006b. Quantitative analysis of β-Sitosterol oxides induced in vegetable oils by natural sunlight, artificially generated light and irradiation. J. Agric. Food Chem., 54: 5410-5415.